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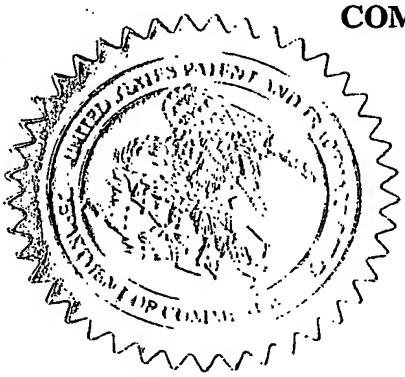

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A/PROV

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV036289757US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Jan	Muzikar	Bloomington, IN
Yehia	Mechref	Bloomington, IN
Milos	Novotny	Bloomington, IN

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHOD AND COMPOSITION FOR ASSAYING MONOSACCHARIDES

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 11
☒ Drawing(s) *Number of Sheets* 2
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 10-0435
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FILING FEE AMOUNT ($) $160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.
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Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Bradford G. Addison

TELEPHONE (317)-231-7253

Date 03/20/2003

REGISTRATION NO. *(if appropriate)* 41,486

Docket Number: 32993-72609

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

BARNES & THORNBURG

11 South Meridian Street
Indianapolis, IN 46204
(317) 236-1313
(317) 231-7433 Fax

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group:	Unknown
Confirmation No.:	Unknown
Application No.:	Unknown
Invention:	Method and Composition for Assaying Monosaccharides
Applicant:	Muzikar et al.
Filed:	Herewith (March 20, 2003)
Attorney Docket:	32993-72609
Examiner:	Unknown

CERTIFICATE UNDER 37 C.F.R. § 1.10

Assistant Commissioner for Patents
Washington, D.C. 20231
Box Provisional Patent Application

Express Mail Label Number: EV036289757US

Date of Deposit: March 20, 2003

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Respectfully submitted,

BARNES & THORNBURG

Bradford G. Addison

BGA/wlb
Indianapolis, IN
(317) 231-7253

PROVISIONAL PATENT APPLICATION

of

Jan Muzikar
Yehia Mechref
and
Milos V. Novotny

For

METHOD AND COMPOSITION FOR ASSAYING MONOSACCHARIDES

Attorney Docket No. 32993-72609
ARTI Docket No. 0371

METHOD AND COMPOSITION FOR ASSAYING MONOSACCHARIDES

FIELD OF THE DISCLOSURE

The present disclosure generally relates to a method and composition for assaying monosaccharides. The present disclosure particularly relates to a method and composition for assaying monosaccharides associated with a glycoprotein utilizing capillary electrophoresis and laser induced fluorescence.

BACKGROUND OF THE DISCLOSURE

When polysaccharides are released from glycoproteins and then separated into their constituent monosaccharides for subsequent analysis of the monosaccharides by capillary electrophoresis with laser-induced fluorescence detector (CD/LIF), the resulting electropherogram may show by-product peaks. These peaks are a problem especially when they appear near the locations where peaks for the monosaccharides are expected. Accordingly, it is desirable to provide an approach which enhances an investigators ability to analyze monosaccharides with, for example, capillary electrophoresis with laser-induced fluorescence detector (CD/LIF).

SUMMARY OF THE DISCLOSURE

A method, composition, and reducing reagent for assaying monosaccharides, for example, monosaccharides associated with a glycoprotein, in accordance with the present disclosure comprises one or more of the following features or combinations thereof:

The composition for assaying monosaccharides may be utilized with capillary electrophoresis and laser induced fluorescence. In particular, the composition for assaying monosaccharides may be utilized to determine the presence and, optionally, the relative concentration of monosaccharides associated with a glycoprotein.

The composition can include a glycoprotein. The composition may also include monosaccharides released from the glycoprotein. The monosaccharides released from the glycoprotein can be associated or derivatized with a fluorophor. For example, one fluorophor the monosaccharides can be associated with is 8-aminopyrene-1,3,6-trisulfonic acid (APTS). The 8-

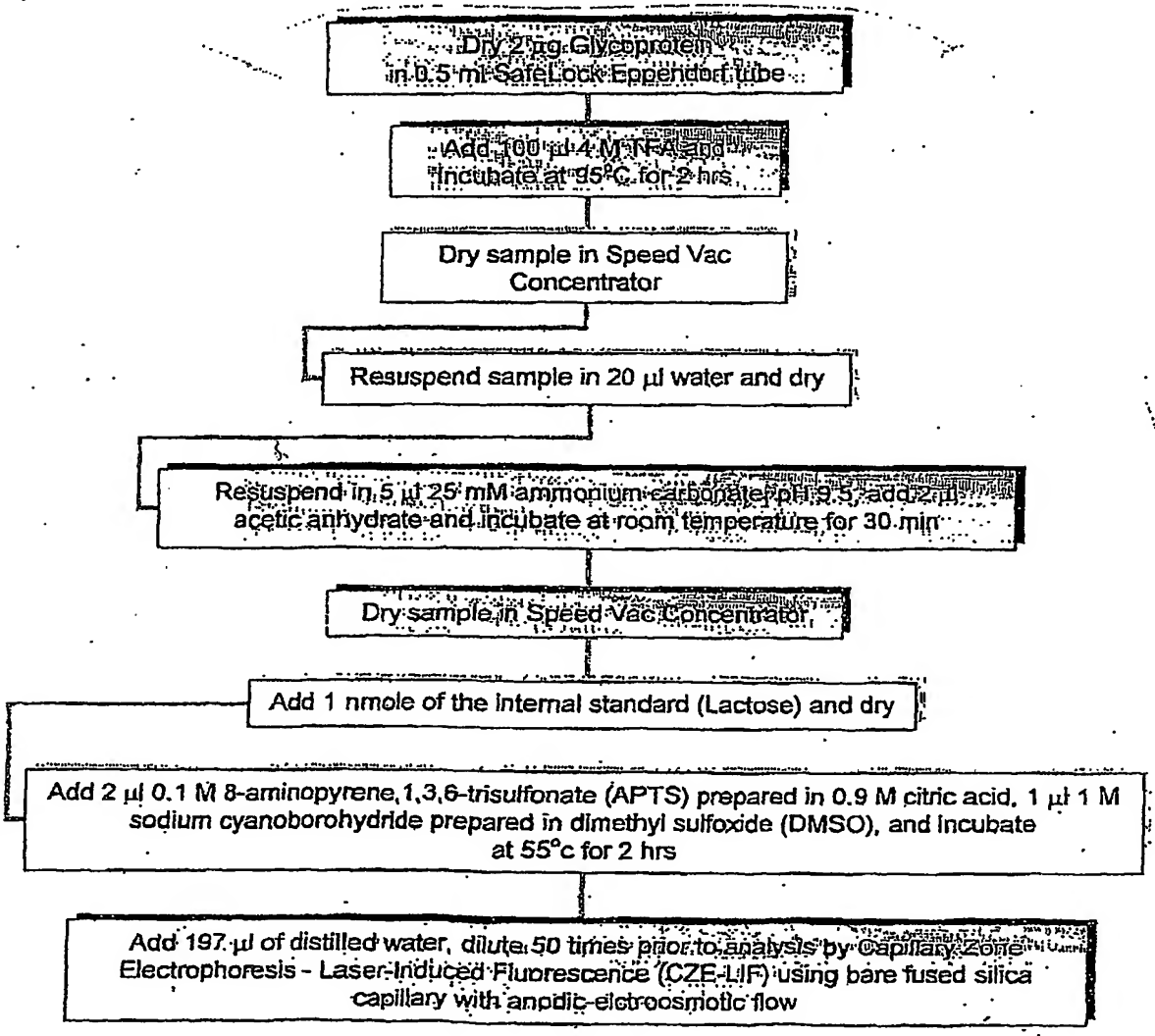
aminopyrene-1,3,6-trisulfonic acid can be prepared in citric acid. The composition can also include a reducing agent in an organic solvent. For example, the organic solvent can include dimethyl sulfoxide (DMSO) and the reducing agent can include sodium cyanoborohydride.

The method for assaying monosaccharides can be utilized to analyze, for example, monosaccharides associated with a glycoprotein. In particular, the method may be utilized to identify monosaccharides associated with a glycoprotein. The method may also be utilized to determine the relative concentrations of the monosaccharides associated with the glycoprotein.

The method may include incubating the glycoprotein in a TFA solution to produce a first incubation product. For example, the glycoprotein can be incubated in a relatively weak solution of TFA at an elevated temperature to produce the first incubation product. In particular, the relatively weak TFA solution can be about 0.1 M TFA. The method can also include incubating the first incubation product with N-acetylneuraminic aldolase to produce a second incubation product. For example, the first incubation product can be incubated with *E. Coli* N-acetylneuraminic aldolase at about room temperature in a phosphate buffer at a pH of about 7.5 to produce the second incubation product. The second incubation product can be incubated in a TFA solution to produce a third incubation product. For example, the second incubation product can be incubated with a relatively strong TFA solution at an elevated temperature to produce the third incubation product. The relatively strong solution of TFA can be about 4 M TFA. The method can further include incubating the third incubation product with ammonium carbonate and acetic anhydrate to produce a fourth incubation product. For example, the ammonium carbonate can be about 25 mM. The method can further include incubating the fourth incubation product in 8-aminopyrene-1,3,6-trisulfonic acid (APTS) with citric acid, at about 0.9 M, and sodium cyanoborohydride, at about 1 M, in dimethyl sulfoxide (DMSO) at an elevated temperature to produce a fifth incubation product. It should be appreciated that the presence of DMSO suppresses the formation of by-product peaks (ghost peaks) which can interfere with a capillary electrophoresis with laser induced fluorescence detector analysis. Accordingly, the fifth incubation product can be utilized in capillary electrophoresis where, for example, laser induced fluorescence detector is used to identify the monosaccharides and their relevant concentrations.

The reducing reagent for assaying monosaccharides may be utilized with capillary electrophoresis and laser induced fluorescence. In particular, the reducing reagent for assaying monosaccharides may be utilized to determine the presence and, optionally, the relative concentration of monosaccharides associated with a glycoprotein. The reducing reagent may include sodium cyanoborohydride prepared in dimethyl sulfoxide (DMSO).

A method for assaying monosaccharides, for example, monosaccharides associated with a glycoprotein, in accordance with the present disclosure comprises one or more features of the following flow chart, or combinations thereof:



Additional features of the present disclosure will become apparent to those skilled in the art upon consideration of the following detailed description of preferred embodiments exemplifying the best mode of carrying out the subject matter of the disclosure as presently perceived.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow chart of an exemplary embodiment of a method for assaying monosaccharides, for example, monosaccharides associated with a glycoprotein, in accordance with the present disclosure; and

Fig. 2 is an electropherogram of APTS labeled monosaccharides prepared using DMSO (Example 1) and THF (Example 2). Note that the by-product peak is assigned with an asterisk. Peaks: (1) N-acetyl galactosamine (GalNAc) , (2) N-acetylmannosamine (ManNAc), (3) N-acetylglucosamine, (4) mannose (Man), (5) glucose (Glc), (6) Fucose (Fuc), and (7) galactose (Gal)

DETAILED DESCRIPTION OF THE DISCLOSURE

While the disclosure is susceptible to various modifications and alternative forms, specific embodiments will herein be described in detail. It should be understood, however, that there is no intent to limit the disclosure to the particular forms described, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the disclosure.

An exemplary flow chart for practicing one method of the present disclosure and for making the reagent is described in Figure 1.

Comparative examples are shown in Figure 2 where a line represents each of Examples 1 and 2, while the inset is an enlargement of the section of interest. Each line is marked for the corresponding Example at the left edge of the figure.

Example 1 shows CE/LIF assay results for a one-pot glycoprotein sample processed according to the present disclosure wherein heating was done in an oven.

Example 2 shows CE/LIF assay results for a one-pot glycoprotein sample process with tetrahydrofuran (THF) used as an organic solvent for the reducing agent instead of DMSO and wherein heating was done in an oven.

The sample solution in each Example was identical and included each of the 7 sugars that can be expected to be encountered in glycoproteins.

(The peak at about 8.5 minutes in each example is the result of a lactose reference maker.)

At about 9.7 minutes in Example 2 the problem by-product peak is seen. As illustrated in Figure 2, this by-product peak makes it difficult to identify the peak for the monosaccharide ManNAc, which, if present, may overlap the by-product peak. Example 1 shows the result of selecting DMSO as an organic solvent for the reducing agent rather than, for example, the THF. Accordingly:

1. In one aspect the invention is a sample preparation for use with capillary electrophoresis and laser-induced fluorescence in an assay to determine the presence and, optionally, the relative concentrations of monosaccharides associated with a glycoprotein, the sample preparation comprising:

(a) the glycoprotein;

(b) monosaccharides released from the glycoprotein with which at least one fluorophor has been associated; and

(c) a reducing agent in an organic solvent

wherein the organic solvent is dimethyl sulfoxide (DMSO).

The fluorophor is, preferably, 8-aminopyrene-1,3,6-trisulfonic acid (APTS) prepared in citric acid and the reducing agent is, preferably, sodium cyanoborohydride.

2. In another aspect the invention is a method for preparing a glycoprotein for identification of the monosaccharides it carries and, optionally, for determining their relative concentrations, the method comprising:

(a) Incubating the glycoprotein with a relatively weak solution of TFA (preferably about 0.1 M TFA) at an elevated temperature to produce a first incubation product;

(b) Incubating the first incubation product with *E. Coli* N-acetylneuraminic aldolase at room temperature in a phosphate buffer at an elevated pH (preferably about pH 7.5) to provide a second incubation product;

(c) Incubating the second incubation product with a relatively strong concentration of TFA (preferably about 4M TFA) at an elevated temperature to produce a third incubation product;

(d) Incubating the third incubation product in ammonium carbonate (preferably at a concentration of about 25 mM) and acetic anhydrate at room temperature to produce a fourth incubation product; and

(e) Incubating the fourth incubation product in APTS prepared in citric acid (preferably at a concentration of about 0.9 M) and in sodium cyanoborohydride (preferably at a concentration of about 1M) prepared in DMSO at elevated temperature to provide a fifth reaction product suitable for use in capillary electrophoresis wherein laser induced fluorescence detector is used to identify the monosaccharides and their relevant concentrations.

3. In yet another aspect the invention is a reducing reagent for use in preparing mixtures of monosaccharides for analysis by capillary electrophoresis using laser induced fluorescence, the reagent comprising sodium cyanoborohydride prepared in DMSO.

While the disclosure has been illustrated and described in detail in the foregoing description, such an illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only the illustrative embodiments have been described and that all changes and modifications that come within the spirit of the disclosure are desired to be protected.

CLAIMS

1. A composition for use in the analysis of monosaccharides, the composition comprising:

a monosaccharide derivatized with a fluorophor; and

a reducing agent in an organic solvent, wherein the organic solvent includes dimethyl sulfoxide.

2. A method for treating monosaccharides in a one-pot preparation, comprising:

subjecting a monosaccharide to a reducing agent in a solvent that contains dimethyl sulfoxide.

3. A method for treating monosaccharides, comprising:

reducing a monosaccharide in dimethyl sulfoxide to cause the suppression of by-product peaks detectable by a laser-induced fluorescence detector.

METHOD AND COMPOSITION FOR ASSAYING MONOSACCHARIDES

ABSTRACT OF THE DISCLOSURE

A method, composition, and reducing reagent for assaying monosaccharides, for example, monosaccharides associated with a glycoprotein, is described.

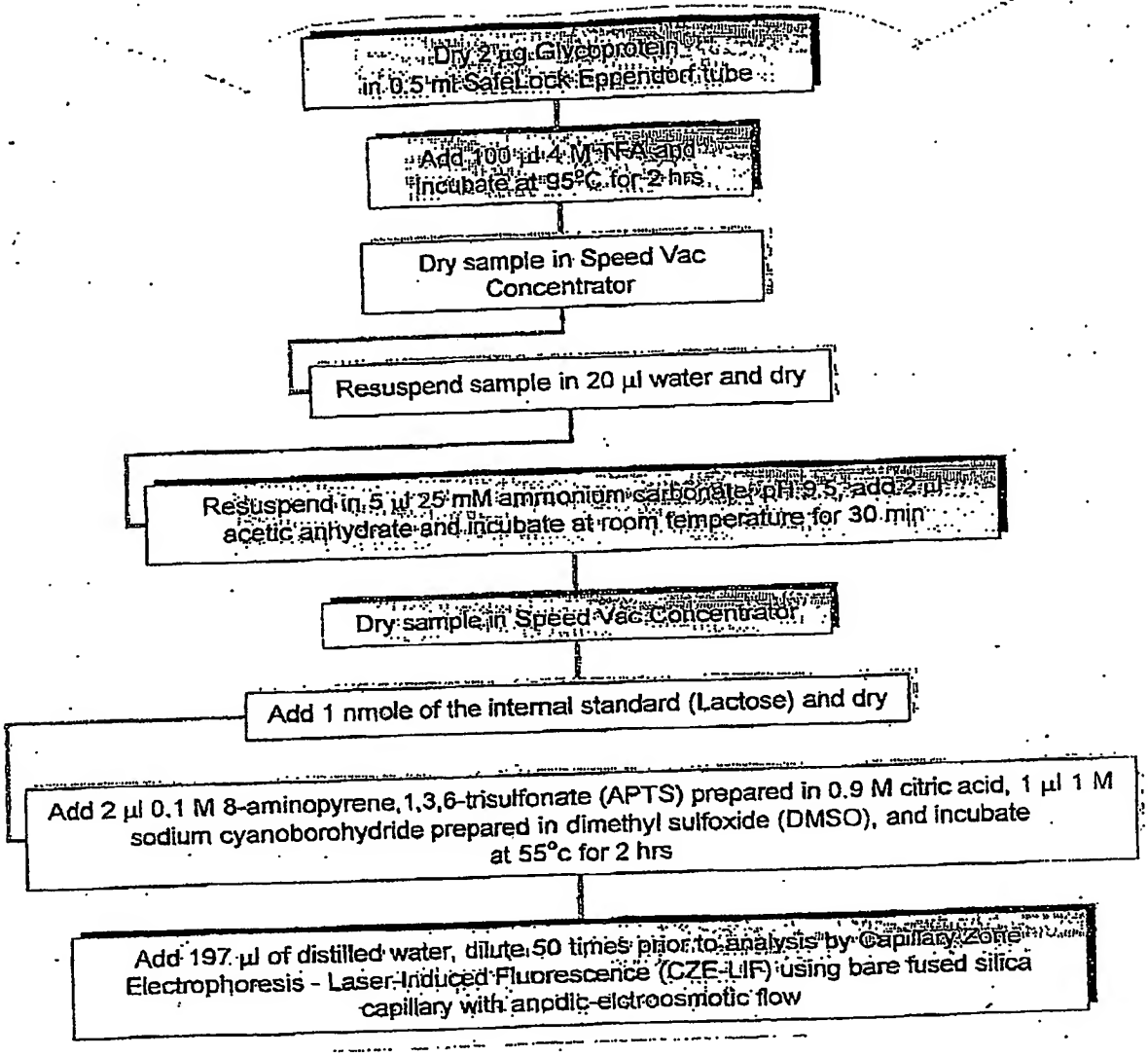

Dry 2 ng Glycoprotein in 0.5 ml SafeLock Eppendorf tube
Add 100 µl 4 M TFA and incubate at 95°C for 2 hrs
Dry sample in Speed Vac Concentrator
Resuspend sample in 20 µl water and dry
Resuspend in 5 µl 25 mM ammonium carbonate, pH 9.5, add 2 µl acetic anhydrate and incubate at room temperature for 30 min
Dry sample in Speed Vac Concentrator
Add 1 nmole of the internal standard (Lactose) and dry
Add 2 µl 0.1 M 8-aminopyrene,1,3,6-trisulfonate (APTS) prepared in 0.9 M citric acid, 1 µl 1 M sodium cyanoborohydride prepared in dimethyl sulfoxide (DMSO), and incubate at 55°c for 2 hrs
Add 197 µl of distilled water, dilute 50 times prior to analysis by Capillary Zone Electrophoresis - Laser Induced Fluorescence (CZE-LIF) using bare fused silica capillary with anodic electroosmotic flow

FIG. 1

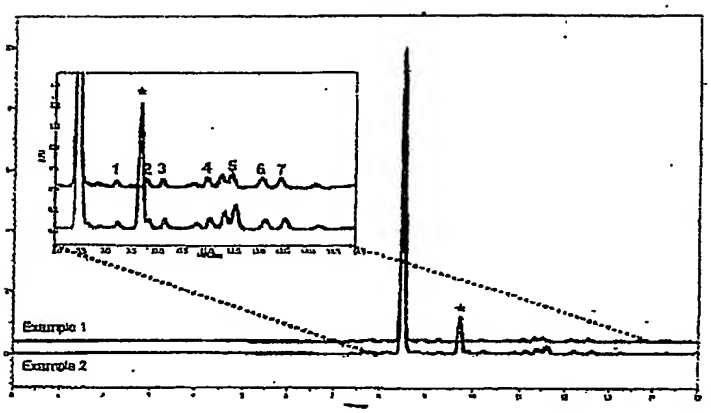


FIG. 2

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